Molluscan Research **19(2):** 31-42 (1998)

Population genetic structure of the muricid gastropod *Lepsiella vinosa* in Gulf St Vincent, South Australia

Lianos Triantafillos [1, 2], *Stephen Donnellan* [3] and *Alan J. Butler* [1, 4]

1 Department of Zoology, University of Adelaide, North Terrace, Adelaide, SA 5000
2 *Present address:* South Australian Aquatic Science Centre, GPO Box 120 Henley Beach, SA 5022
3 South Australian Museum, Division of Natural Science, North Terrace, Adelaide, SA 5000
4 *Present address:* CSIRO Division of Marine Research, GPO Box 20, North Beach, WA 6020

Abstract

Allozyme electrophoresis was used to examine the population genetic structure of the marine gastropod *Lepsiella vinosa*, which produces benthic egg capsules with crawling juveniles. Samples of *L. vinosa*, taken from five locations along the coast of Gulf St Vincent, South Australia, including replicate samples within three of the locations, were examined for allozyme variation at four polymorphic loci (*Acon-1, Got, Gpi* and *Lap*). Genotypic frequencies were consistent with expectations under conditions of Hardy-Weinberg equilibrium. Genetic divergence between samples separated by only 500 m was small, in contrast to the great divergence found between samples separated by more than 10 km. This degree of genetic divergence ($F_{ST} = 0.200 \pm 0.073$; Nei's D mean = 0.071) indicated low gene flow ($N_em = 1.08$ immigrants per generation) over distances $\geq$ 10 km. The data are consistent with a 'discrete subpopulation model'.

Key words : population, genetic structure, *Lepsiella vinosa*, South Australia.

Introduction

The dispersal ability of sedentary benthic marine invertebrates is largely predicted by their mode of larval development. Species with extensive dispersal, such as those with long planktonic larval stages, tend to have a broad distribution and little genetic differentiation among populations over large distances (Scheltema 1971, 1986; Hedgecock 1986; Janson 1987; Ovenden *et al.* 1992; Parsons 1996). In contrast, species with limited dispersal, such as those lacking a planktonic larval phase, tend to have a restricted distribution and high levels of genetic differentiation between populations (Snyder & Gooch 1973; Crisp 1978; Burton 1983; Johnson & Black 1984; Grant & Utter 1988). In these latter species, allele frequencies can be significantly different in populations separated by only a few hundred metres (Janson & Ward 1984; Grant & Utter 1988; Carvalho & Piertney 1997).

There are, however, several notable exceptions. For example, some brooding invertebrates that lack a planktonic larval stage are more widespread than related species that have a planktonic dispersive phase (Highsmith 1985; Johannesson 1988; Martel & Chia 1991). This implies that brooding invertebrates have alternative mechanisms for dispersal. One possible such mechanism is dispersal by attachment to floating objects, *i.e.* rafting (O'Foighil 1989; Martel & Chia 1991). Indeed, several authors have suggested that rafting of sedentary benthic invertebrates with direct development may be a more powerful means of long distance dispersal than is larval diffusion (Dell 1972; Jackson 1986; Johannesson 1988). Another potential dispersal mechanism is floating. Although dispersal by floating is generally limited to a local scale and is restricted to smaller invertebrates, it does increase the probability of rafting and thus long distance dispersal (Highsmith 1985).

A second notable exception to the relationship between larval development and geographic distribution is the presence of population subdivision in species with planktonic larvae (Burton 1983; Hedgecock 1986; O'Foighil 1989; Parsons 1996). This subdivision may be driven by selective forces, which vary between age classes and from habitat to habitat (e.g. Koehn *et al.* 1976), or it may be attributable to a variety of biological or physical processes that restrict dispersal (reviewed by Hedgecock 1986).

Lepsiella vinosa (Lamarck 1882) is a dioecious, muricid gastropod found abundantly in the intertidal region along the coastline of southern Australia from NSW to Albany, WA (Macpherson & Gabriel 1962; Wilson & Gillet 1974). Unlike most marine gastropods, this species lays gelatinous egg capsules which hatch to liberate crawling juveniles (Synnot 1980; Smith *et al.* 1989). This mode of development coupled with the relatively sedentary behaviour of the adults limits their potential for dispersal. The wide geographical range of *L. vinosa* contrasts with its apparent limited dispersal ability, making it a noteworthy candidate for examination. The main objective of the present study was to examine the genetic differentiation of this species and thus infer its dispersal ability.

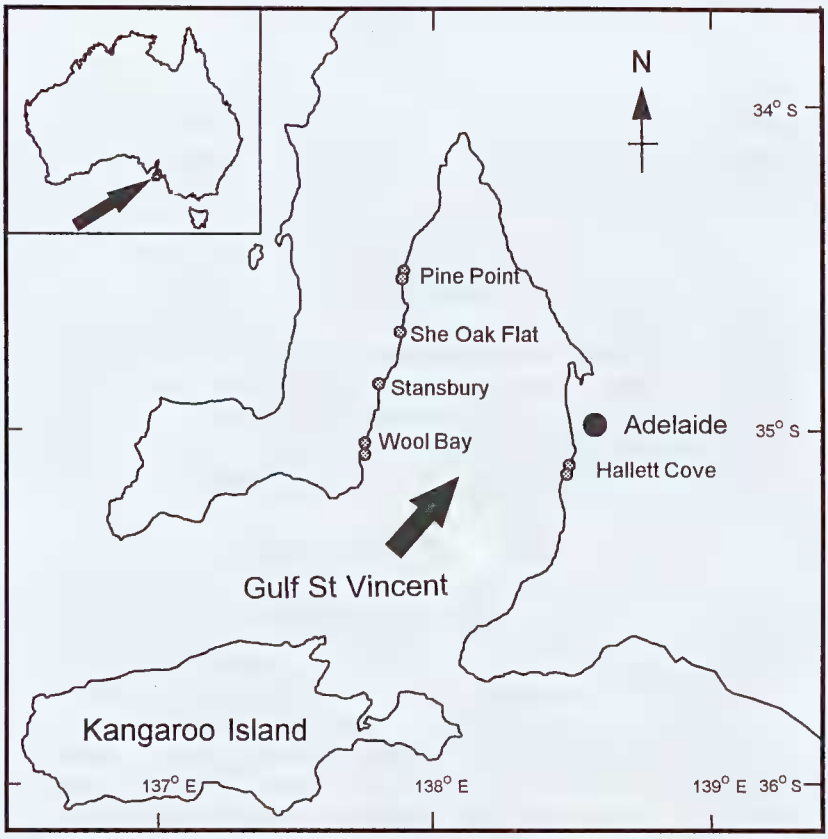

Figure 1. Map showing collection sites in Gulf St Vincent , South Australia

Materials and Methods

Collection of samples

Samples of *L. vinosa* were collected from five intertidal locations, 10-80 km apart, along the coast of Gulf St Vincent, during November 1992 and January 1993 (Fig. 1).

At the same time, additional samples were collected at Hallett Cove, Wool Bay and Pine Point from sites less than 500 m from the original collection. These additional samples were used to measure genetic variation within a location (*i.e.* at a spatial scale smaller than the span of the location). At each site, individuals were randomly collected from the smallest area possible, usually from an area less than 3 m^2. Each sample was kept in a separate tank filled with aerated sea water, for a minimum of ten days without food, to eliminate the possibility of spurious electrophoretic patterns due to gut contents.

Allozyme electrophoresis

Materials and methods used for electrophoresis follow the procedures of Richardson *et al.* (1986). The shell was carefully crushed and the hepatopancreas dissected out. The hepatopancreas was homogenised in an equal volume of lysis buffer (0.01% NADP, 0.01% 2-mercaptoethanol, distilled H_2O). Homogenates were centrifuged at 10,000 g for 10 minutes, and the supernatant transferred into separate capillary tubes and stored at -80°C, pending electrophoresis. Interpretations of gels were made with reference to the predicted quaternary structure of the enzyme and band patterns of putative heterozygotes (Richardson *et al.* 1986).

A pilot study was conducted by screening 40 enzyme systems on 40 individuals (5 from each sample) to identify polymorphic loci and to optimise electrophoretic conditions. Four polymorphic loci (*Acon -1, Got, Gpi* and *Lap*) were screened (Table 1).

Table 1. Enzyme Commission (E.C.) numbers and buffer conditions of the polymorphic enzymes screened.

Enzyme name	Locus	E.C. number	Number of alleles	Buffer system (after Richardson *et al.* 1986).
Aconitate hydratase	*Acon - 1*	4.3.1.3	3	0.02M Phosphate pH 7.0
Aspartate aminotransferase	*Got*	2.6.1.1	2	0.05M Tris Maleate pH 7.8
Glucose phosphate isomerase	*Gpi*	5.3.1.9	2	0.02M Phosphate pH 7.0
Leucine aminopeptidase	*Lap*	3.4.11.1	2	0.01M Citrate Phosphate pH 6.4

As well as having mobility controls on every gel, approximately 10% of all individuals, randomly chosen, were retyped at each locus as a check for scoring consistency. Allozyme phenotypes of putative heterozygotes at the four polymorphic loci were consistent with those expected on the basis of the predicted quaternary structure of the enzymes.

Statistical analysis

Allelic frequencies were calculated for each locus for each of the samples using the BIOSYS-1 program (Swofford & Selander 1981). Locus x locus χ^2 homogeneity tests were used to test the assumption that loci were in linkage equilibrium (Hill 1974). The genotype frequency distribution of each polymorphic locus in each sample was tested for goodness-of-fit to Hardy-Weinberg expectations using a χ^2 test (Siegel 1956) with Levene's (1949) correction for small sample sizes.

Multilocus measures of genetic variability calculated within each sample were:

- **a**, mean number of alleles per locus;
- **P**, the proportion of polymorphic loci whose most common allele is not greater than 0.95 in frequency;
- $\mathbf{H_o}$, the mean heterozygosity per locus observed by direct count of individuals;
- $\mathbf{H_e}$, the unbiased estimate of mean heterozygosity per locus expected under Hardy-Weinberg equilibrium.

The methods for calculation of these measures are given in Nei (1978). Heterozygote deficiencies from Hardy-Weinberg expectations were analysed for each sample using the fixation index of Wright (1978).

Pairwise χ^2 tests for heterogeneity of allele frequencies among locations were performed for all loci (Siegel 1956). The sequential Bonferroni test was used to adjust probabilities for these tests (Rice 1989). The overall genetic differentiation between samples was calculated as Nei's (1978) unbiased measure of genetic distance. The results were represented diagrammatically as a dendrogram in which clustering was determined by the unweighted pair-group method algorithm (UPGMA; Sokal & Sneath 1963). The dendrogram is not intended to reflect phylogenetic relationships, but rather to give a visual indication of the genetic diversity between samples.

For each locus, the magnitude of the allelic variation occurring within location (F_{IS}) and between locations (F_{ST}; Wright 1978) was calculated using the DIPLOID program (Weir 1990). An estimate of the variance of both F_{IS} and F_{ST} was obtained by jackknifing loci, as described by Weir & Cockerham (1984), to obtain 95% confidence limits (DIPLOID; Weir 1990). The significance of F_{IS} and F_{ST} values were tested with χ^2 tests, using equations in Waples (1987). Based on the island model of Wright (1978), values of gene flow among locations were estimated from F_{ST} using the equation $N_em = [(1/F_{ST}) - 1] / 4$, where N_e is the effective population size and m is the migration rate. Estimates of the variance of the mean number of immigrants per generation were obtained by jackknifing loci, to obtain 95% confidence limits for Weir & Cockerham's (1984) F_{ST} and then calculating gene flow N_em from these.

Results

Allele frequencies for each locus for each sample are presented in Table 2. Two alleles were observed at *Got*, *Gpi* and *Lap*, and three alleles at *Acon-1*. *Gpi* and *Got* allele frequencies were dominated in all samples by one allele, while *Acon-1* and

Lap were more variable. *Lap*^{b} had the largest difference in frequency between samples, ranging from 0.36 at Hallett Cove 2 to 0.99 at Pine Point 1. There was no fixed difference between samples as they all shared at least one allele at each locus. The She Oak Flat sample possessed an allele, *Acon-1*^{c} (at a frequency of 0.09), which was absent from other samples.

Table 2. Allele frequencies for *L. vinosa* at four polymorphic loci

Locus	allele	Wool Bay 1	Wool Bay 2	Stansbury	She Oak Flat	Pine Point 1	Pine Point 2	Hallett Cove 1	Hallett Cove 2
Acon - 1	c				0.09				
	b	0.54	0.41	0.50	0.27	0.11	0.21	0.07	0.24
	a	0.46	0.59	0.50	0.64	0.89	0.79	0.93	0.76
Got	b	0.01	0.08			0.10	0.05	0.06	0.06
	a	0.99	0.92	1.00	1.00	0.90	0.95	0.94	0.94
Gpi	b	0.03	0.06	0.04	0.26	0.06	0.04		
	a	0.97	0.94	0.96	0.74	0.94	0.96	1.00	1.00
Lap	b	0.71	0.79	0.49	0.42	0.99	0.93	0.40	0.36
	a	0.29	0.21	0.51	0.58	0.01	0.07	0.60	0.64
	N	57	86	34	81	84	79	87	91

Alleles were labelled alphabetically in order of increasing mobility. Multiple loci encoding the same enzyme (isoenzyme) were designated by consecutive numbers, with '1' denoting the slowest migrating isoenzyme.
N = number of individuals typed.

Locus x locus χ^2 homogeneity tests indicated no linkage disequilibrium ($p>0.05$) between any loci within the eight samples. Therefore each of the four polymorphic loci could be treated as independent tests of the relationship between the samples.

Heterozygosity (H_o, H_e) and the proportion of polymorphic loci varied substantially between samples (Table 3). The proportion of polymorphic loci per sample varied between 50 and 100% with an average of 71%. In addition, levels of mean observed heterozygosity (H_o) were also high (H_o mean = 0.210; H_o range: 0.119-0.312).

The observed heterozygosity (H_o) for each sample conformed with Hardy-Weinberg expectations. Only one significant departure from Hardy-Weinberg expectations was detected, a heterozygote deficit in *Acon-1* at Stansbury ($F = 0.529$). The results for heterozygosity were consistent with χ^2 tests of fit to Hardy-Weinberg expectations for individual loci. Again, there was only one significant departure ($\chi^2 = 10.07$, $p<0.001$) from Hardy-Weinberg equilibrium, namely for *Acon-1* at Stansbury. As there was no deviation from Hardy-Weinberg expectations at samples within locations, the respective replicate samples were pooled and the resulting three populations subjected to further goodness-of-fit tests. All three populations at Wool Bay, Pine Point and Hallett Cove conformed to Hardy-Weinberg expectations.

Table 3. Measures of genetic variability at 4 polymorphic loci for all samples

Site	Mean numbers of alleles per locus	Percentage of polymorphic loci*	Direct count	Hardy Weinberg expected**
	a	P	H_o	H_e
Wool Bay 1	2.0 ± 0.0	50	0.215 ± 0.105	0.246 ± 0.124
Wool Bay 2	2.0 ± 0.0	100	0.247 ± 0.070	0.270 ± 0.087
Stanbury	1.8 ± 0.3	50	0.206 ± 0.109	0.275 ± 0.135
She Oak Flat	2.0 ± 0.4	75	0.312 ± 0.109	0.349 ± 0.120
Pine Point 1	2.0 ± 0.0	75	0.119 ± 0.042	0.125 ± 0.042
Pine Point 2	2.0 ± 0.0	75	0.152 ± 0.048	0.158 ± 0.059
Hallett Cove 1	2.0 ± 0.3	75	0.204 ± 0.120	0.183 ± 0.104
Hallett Cove 2	1.8 ± 0.3	75	0.223 ± 0.100	0.235 ± 0.107
Mean	1.9 ± 0.16	71.9	0.210 ± 0.087	0.230 ± 0.097

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95
** Unbiased estimate (see Nei, 1978)

Table 4. F-statistics describing within population structuring (F_{IS}), between population structuring (F_{ST}) and gene flow (N_em) among all samples and only samples from the western coastline of Gulf St Vincent.

	All samples		Western coastline samples	
Locus	F_{IS}	F_{ST}	F_{IS}	F_{ST}
Acon -1	0.141***	0.129***	0.159**	0.117***
Got	-0.039^{ns}	0.021**	-0.21^{ns}	0.037***
Gpi	0.096*	0.123**	0.099*	0.099**
Lap	0.017^{ns}	0.287***	0.070^{ns}	0.274***
Over all loci	0.070^{ns}	0.187***	0.100^{ns}	0.163***
Unbiased F_{ST} ± 95%	0.074 ± 0.5	0.2 ± 0.073	0.11 ± 0.044	0.167 ± 0.06
N_em (range)		1.08 (0.66 - 1.72)		1.28 (0.9 - 2.01)

* P<0.05; ** P<0.01; *** P<0.001; ns, not significant

Many of the χ^2 tests for heterogeneity of allele frequencies were significant at the Bonferroni-adjusted level of 0.00045 (0.05/112). These tests indicated that the She Oak Flat location possessed the most divergent allelic array because it had the largest number of significantly heterogeneous χ^2 tests when compared to the other location. In contrast, there was no significant difference in allelic frequencies within Wool Bay and Pine Point. At Hallett Cove, only *Acon-1* was significant at the $P< 0.00045$ level.

The overall pattern of genetic differentiation of samples was calculated as Nei's (1978) unbiased measure of genetic distance (Table 4). Genetic differentiation was smallest between samples from the same location *ie.* Wool Bay, Pine Point and Hallett Cove (*D* mean = 0.006; *D* range 0.004 to 0.008). Any pairwise comparisons between two locations resulted in much higher values of genetic distance. The mean genetic distance for all possible pairwise comparisons between locations was 0.071, with a range of 0.013 to 0.137. This differentiation was an order of magnitude higher than between pairs of samples from the same location. The values of genetic distance are presented in a dendrogram (Figure 2).

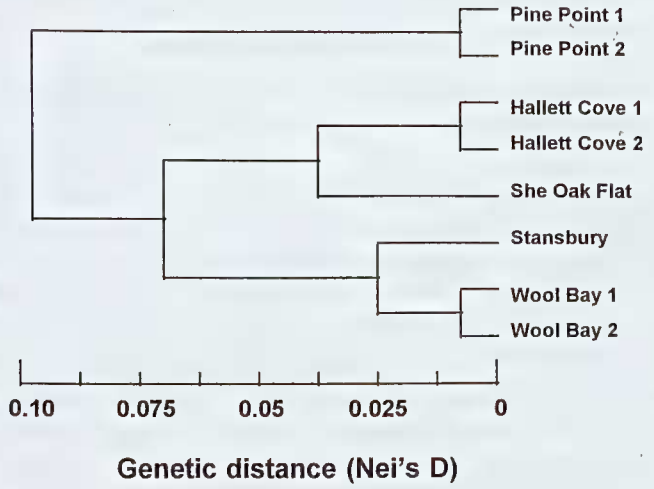

Figure 2. Dendrogram based on Nei's (1978) genetic distance for all samples constructed by UPGMA clustering (Sokal & Sneath 1963)

From the dendrogram, it can be clearly seen that the samples collected within the same location had the highest genetic similarity. Apart from this, and the close geographic proximity and genetic similarity of Stansbury to Wool Bay, clustering did not correspond to that expected from spatial distance alone. For example, according to the dendrogram, She Oak Flat was more similar to Hallett Cove than to its geographically closest neighbour at Pine Point. A pairwise comparison of genetic distance with geographic separation between all combinations of locations also illustrated no clear correlation (Fig. 3). Furthermore, a comparison of allele frequencies, with respect to geographic locations, lacked any apparent trends such as clines, with the fluctuations appearing to be chaotic.

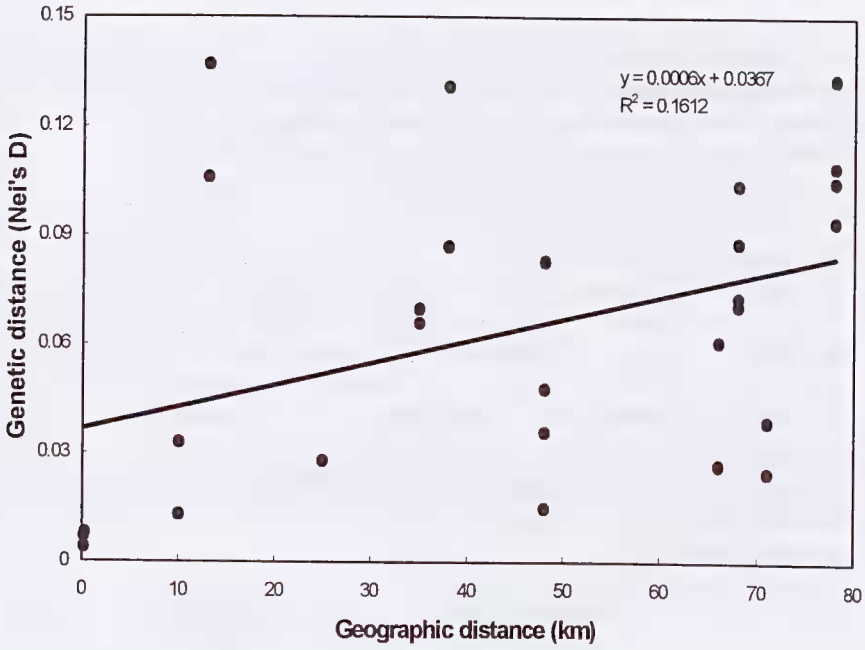

Figure 3. Genetic distance (Nei's *D*) as a function of geographic distance (km; straight line) between pairwise comparison of samples.

Two F_{IS} values for individual loci (*Acon-1* and *Gpi*) were significant, indicating possible within-location substructuring (Table 4). However, the F_{IS} value for *Gpi* was only marginally significant ($F_{IS} = 0.096$, $p<0.05$), and the value for *Acon-1* ($F_{IS} = 0.141$, $p<0.001$) was biased due to the large contribution of variation from the Stansbury location. In addition, the mean F_{IS} value over all loci was non-significant ($F_{IS} = 0.07$). This, and the fact that the detailed χ^2 tests of individual loci failed to demonstrate any significant departures from Hardy-Weinberg expectations (except for *Acon-1* at Stansbury), suggested that within-location substructuring, if present, was below the sensitivity of our methods.

Weir & Cockerham's (1984) F_{ST}, which take into account variation in sample sizes, demonstrated significant differences in allele frequencies among locations for all loci (Table 4). Differentiation among all locations, measured by F_{ST}, ranged from 0.021 for *Got* to 0.287 for *Lap*, with an average for all loci of 0.187 ($p<0.001$). When Hallett Cove was excluded from this analysis, leaving only the locations on the western coastline of the gulf, the genetic differentiation amongst locations ($F_{ST} = 0.163$; $p<0.001$) was only slightly lower than that of the total Gulf St Vincent data set. This indicated that most of the genetic variation was derived from between locations and not from between coastlines.

Estimates of gene flow indicated very low levels of exchange between locations ($N_em = 1.08$; range 0.66 to 1.72 immigrants per generation). Excluding the geographically furthest location (Hallett Cove) from the analysis still resulted in N_em values ($N_em = 1.28$; range 0.9 to 2.01 immigrants per generation) similar to other species of marine gastropods with direct development (Hoskin 1996).

Discussion

The genetic population structure of *L. vinosa* was consistent with the predicted effects of recruitment via sexually produced offspring with a low dispersal ability. Genotypic frequencies within samples were for the most part in good agreement with expectations for Hardy-Weinberg equilibrium. Only *Acon-1* at Stansbury exhibited a departure from Hardy-Weinberg expectations, attributable to a significant heterozygote deficit.

Heterozygote deficiency is a common phenomenon in molluscs (Zouros & Foltz 1984; Volckaert & Zouros 1989). Possible explanations include typing errors, non-random mating, selection, the presence of null alleles and the Wahlund effect (Singh & Green 1984; Hare *et al.* 1996). As the phenotypes for *Acon-1* were clear and readily resolved, typing errors were improbable. Non-random mating due to inbreeding or assortative mating was also considered unlikely, because either of these processes would be expected to give heterozygote deficiencies for all loci in that sample. A common explanation for heterozygote deficiency in molluscs is selection (Zouros & Foltz 1984). However, it is generally restricted to species with high differential mortality (Richardson *et al.* 1986), such as those with long larval phases (e.g. Singh & Green 1984). As *L. vinosa* lacks a planktonic phase it is unlikely that this deficit is the result of selective forces.

Hence, either the presence of null alleles (Milkman & Beaty 1970) or the Wahlund effect (e.g. Koehn *et al.* 1976; Johnson & Black 1984) seem the most likely candidates to explain the observed deficit. Without further analysis, preferably at the DNA level, we cannot discount either of these hypotheses. The absence of linkage disequilibrium coupled with high levels of genetic variation indicates that none of the populations have recently passed through a significant population bottleneck.

In contrast to several studies of marine gastropods with direct development (Johnson & Black 1984; Grant & Utter 1988), intrapopulation allelic frequencies for *L. vinosa* over a range of 500 m were homogeneous. This implies that panmixia prevails over this scale. As this species has no planktonic larval dispersive stage, possible explanations for this genetic cohesion include both adult migration and passive dispersal. The contribution of adult migration to gene flow is likely to be small given the sedentary nature of this species. A possible dispersal mechanism capable of maintaining gene flow levels over this scale is floating. Martel & Chia (1991) found that gastropods similar in size to juvenile *L. vinosa* floated in the water column and, coupled with the ability of this species to breed throughout the year (Synnot 1980), potentially provides a constant source of migrants. Size and weight constraints, vulnerability to predation or starvation and possible advection away from suitable habitats, suggest that floating is likely to be a local-scale dispersal mechanism (Highsmith 1985; Martel & Chia 1991).

In contrast to the homogeneity within locations, there was widespread variation in allelic frequencies for all polymorphic loci between samples separated by more than 10 km. Mean genetic distance values were similar to those for other gastropods with non-pelagic larvae over a similar geographic range (Day & Bayne 1988). Multilocus heterogeneity between samples is

consistent with population subdivision rather than locus-specific effects such as selection (Richardson *et al.* 1986).

Examination of the distributional pattern of allelic variation gives further indications of the degree of isolation between locations. She Oak Flat has an allele (*Acon-1*^{c}) not found elsewhere. This suggests that gene flow between this location and its neighbours, only 13 km distant in the case of Pine Point, is very low or non-existent and has not occurred for a period of time required for alternative allelic accumulations (Larson *et al.* 1984). The pattern of allele frequency distributions is disjunct, there being no clear correlation between genetic and geographic distance.

Our data suggest that the dispersal ability of *L. vinosa* is insufficient to prevent genetic differentiation at locations separated by more than 10 km. This interpretation is supported by very low levels of calculated gene flow, which at $N_em < 1$ can be considered insufficient to prevent differential between locations due to random genetic drift (Slatkin 1985). The genetic population structure of this species is consistent with the discrete subpopulation model of Richardson *et al.* (1986), the population consisting of a series of subpopulations within which mating occurs at random. These subpopulations are separated from one another by restricted dispersal that allows very little migration between them. Our data indicate that the size of subpopulations is somewhere between 500 m and 10 km. Further investigations, at a finer scale, would give a more accurate estimate of the neighbourhood size of subpopulations.

If this population model is correct, then how does *L. vinosa* maintain a wide geographical range? The most likely explanation is rafting, which has been suggested as the main mechanism of long range dispersal in many species of brooding invertebrates (Highsmith 1985; Jackson 1986; Johannesson 1988; Martel & Chia 1991). *L. vinosa* is found in the intertidal region where rafting agents such as drift wood and algal wrack abound. Furthermore, the newly-hatched juveniles of this species are small enough to float on the water surface (see Martel & Chia 1991), increasing the probability of rafting because they are more likely to encounter rafting agents (Highsmith 1985). However, the level of dispersal by rafting, while able to found new populations, is insufficient to maintain allele frequency homogeneity between widely separated locations.

Acknowledgments

This work was supported by the University of Adelaide and the South Australian Museum. We thank Mark Adams for statistical advice and for help during the development of allozyme markers, and Scoresby Shepherd, Andrew Boulton and Rod Connolly for their constructive criticisms of the manuscript. The technical help of the staff at the Evolutionary Biology Unit (SA Museum) was greatly appreciated.

References

Burton, R. S. 1983. Protein polymorphism and genetic differentiation of marine invertebrate populations. *Marine Biology Letters* **4:** 193-206.

Carvalho, G. R., & Piertney, S. B. 1997. Interspecific comparisons of genetic population structure in members of the *Jaera albifrons* species complex. *Journal of the Marine Biological Association of the United Kingdom* **77:** 77-93.

Crisp, D. J. 1978. Genetic consequences of different reproductive strategies in marine invertebrates. Pp. 257-273. In: Battaglia, B. & Beardmore, J. A.*Marine Organisms: Genetics, Ecology and Evolution*. Plenum Press, New York.

Day, A. J., & Bayne, B. L. 1988. Allozyme variation in populations of the dog-whelk *Nucella lapillus* (Prosobranchia: Muricacea) from the South West peninsula of England. *Marine Biology (Berlin)* **99:** 507-513.

Dell, R. K. 1972. Antarctic benthos. *Advanced Marine Biology* **10:** 1-216.

Endler, J. A. 1973. Gene flow and population differentiation. *Science* **179:** 243-250.

Grant, W. S., & Utter, F. M. 1988. Genetic heterogeneity on different geographical scales in *Nucella lamellosa* (Prosobranchia, Thaididae). *Malacologia* **28:** 275-287.

Hare, M. P., Karl, S. A., & Avise, J. C. 1996. Anonymous nuclear DNA markers in the American oyster and their implications for the heterozygote deficiency phenomenon in marine bivalves. *Molecular Biology and Evolution* **13:** 334-345.

Hedgecock, D. 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? *Bulletin of Marine Science* **39:** 550-564.

Highsmith, R. C. 1985. Floating and algal rafting as potential dispersal mechanisms in brooding invertebrates. *Marine Ecology Progress Series* **25:** 169-179.

Hill, W. G. 1974. Estimation of linkage disequilibrium in randomly mating populations. *Heredity* **33:** 229-239.

Hoskin, M. G 1997. Effect of contrasting modes of larval development on the genetic structures of populations of three species of prosobranch gastropods. *Marine Biology (Berlin)* **127:** 647-656.

Jackson, J. B. C. 1986. Modes of dispersal of clonal benthic invertebrates: consequences for species' distribution and genetic structure of local populations. *Bulletin of Marine Science* **39:** 588-605.

Janson, K. 1987. Allozyme and shell variation in two marine snails (Littorina, Prosobranchia) with different dispersal abilities. *Biological Journal of the Linnean Society* **30:** 245-256.

Janson, K., & Ward, R. D. 1984. Microgeographic variation in allozyme and shell characters in *Littorina saxatilis* Olivi (Prosobranchia: Littorinidae). *Biological Journal of the Linnean Society* **22:** 289-307.

Johannesson, K. 1988. The paradox of Rockall: why is a brooding gastropod (*Littorina saxatiliis*) more widespread than one having a planktonic larval dispersal stage (*L. littorea*)? *Marine Biology (Berlin)* **99:** 507-513.

Johnson, M. S., & Black, R. 1984. The Wahlund effect and the geographical scale of variation in the limpet *Siphonaria* sp. *Marine Biology (Berlin)* **79:** 295-302.

Koehn, R. K., Milkman, R., & Mitton, J. 1976. Population genetics of marine pelecypods. IV. Selection , migration and genetic differentiation in the blue mussel, *Mytilus edulis. Evolution* **30:** 2-32.

Larson, A., Wake, D. B., & Yanev, K. P. 1984. Measuring gene flow among populations having high levels of genetic fragmentation. *Genetics* **106:** 293-308.

Levene, H. 1949. On a matching problem arising in genetics. *Annual in Mathematical Statistics* **20:** 91-94.

Macpherson, J.H. & Gabriel, C.J. 1962. *Marine Molluscs of Victoria.* Melbourne University Press, Melbourne.

Martel, A., & Chia, F. 1991. Drifting and dispersal of small bivalves and gastropods with direct development. *Journal of Experimental Marine Biology and Ecology* **150:** 131-147.

Milkman, R., & Beaty, L. D. 1970. Large-scale electrophoretic studies of allelic variation in *Mytilus edulis. Biological Bulletin* **139:** 4-30.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89:** 583-590.

O'Foighil, D. 1989. Planktotrophic larval development is associated with a restricted geographic range in *Lasaea*, a genus of brooding, hermaphroditic bivalves. *Marine Biology (Berlin)* **103:** 349-358.

Ovenden, J. R., Brasher, D. J., & White, R. W. G 1992. Mitochondrial DNA analyses of the red rock lobster (*Jasus edwardsii*) supports an apparent absence of population subdivision throughout Australasia. *Marine Biology (Berlin)* **112:** 319-326.

Parsons, K. E. 1996. The genetic effects of larval dispersal depend on spatial scale and habitat characteristics. *Marine Biology (Berlin)* **126:** 403-414.

Rice, W. R. 1989. Analysing tables of statistical tests. *Evolution* **43:** 223-225.

Richardson, B. J., Baverstock, P. R., & Adams, M. 1986. *Allozyme Electrophoresis: A Handbook of Animal Systematics and Population Structure*. Academic Press, Sydney.

Scheltema, R. S. 1971. Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. *Biological Bulletin (Woods Hole)* **175:** 218-229.

Scheltema, R. S. 1986. On dispersal and planktonic larvae of benthic invertebrates: an electic overview and summary of problems. *Bulletin of Marine Science* **39:** 290-322.

Siegel, S. 1956. *Non Parametric Statistics for the Behavioural Sciences*. McGraw-Hill Kogakusha, Tokyo.

Singh, S.M. & Green, R.H. 1984. Excess of allozyme homozygosity in marine molluscs and its possible biological significance. *Malacologia* **25:** 569-581.

Slatkin, M. 1985. Gene flow in natural populations. *Annual Review in Ecological Systematics* **16:** 393-430.

Smith, B.J., Black, J.H. & Shepherd, S.A. 1989. Molluscan egg masses and capsules. in: Shepherd. S.A. & Thomas, I.M. (eds.). *Marine Invertebrates of Southern Australia, Part II.* pp. 841-862. South Australian Government Printing Divison, Adelaide.

Snyder, T. P., & Gooch J. L. 1973. Genetic differentiation in *Littorina saxatilis* (Gastropoda). *Marine Biology (Berlin)* **22:** 177-182.

Sokal, R. R., & Sneath, P. H. A. 1963. *Principles of Numerical Taxonomy*. W. H. Freeman, San Francisco.

Swofford, D. L., & Selander, R. B. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72:** 281-283.

Synnot, R. N. 1980. The egg capsules of *Lepsiella vinosa* (Lamarck 1882) (Muricidae: Thaidinae). *Journal of the Malacological Society of Australia* **4:** 209-211.

Volckaert, F., & Zouros, E. 1989. Allozyme and physiological variation in the scallop *Placopecten magellanicus* and a general model for the effects of heterozygosity on fitness in marine molluscs. *Marine Biology (Berlin)* **103:** 51-61.

Waples, R. S. 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution* **41:** 385-400.

Weir, B. S. 1990. Genetic Data Analysis. Sinauer, Sunderland, Massachusetts.

Weir, B. S., & Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38:** 1358-1370.

Wilson, B.R & Gillett, K. 1974. *Australian Shells*. Revised edition. A. H. & A. W. Reed, Sydney.

Wright, S. 1978. *Evolution and the Genetics of Populations: Variability Within and Among Populations*. University of Chicago Press, Chicago.

Zouros, E., & Foltz, D. W. 1984. Possible explanations of heterozygote deficiency in bivalves molluscs. *Malacologia* **25:** 583-591.